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Overview

Principles of Cartilage Repair and Regeneration

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An experimental approach and logic are presented for the regeneration of skeletal tissues that focus on the recapitulation of embryonic events starting with an uncommitted progenitor cell population that the authors refer to as mesenchymal stem cells. The repair and regeneration of articular cartilage, which itself has no repair potential, is the subject of this presentation. Full thickness cartilage defects were created in the medial condyle of the distal femur. Self repair (empty defects), articular chondrocytes (allografts), and autologous mesenchymal stem cells were used and the results are reported in selected examples from more than 800 rabbit knees. The optimal number of the appropriate cells delivered in a supportive vehicle to a defect pretreated with a dilute trypsin solution to optimize the integration of repair with normal host cartilage provides a methodology in which regeneration of articular cartilage can be observed. The principles have relevance to the clinical repair and regeneration of cartilage and other skeletal defects.

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The authors suggest as a first principle that the events of embryonic skeletal tissue formation hold the key to understanding and controlling adult tissue repair and regeneration. How do complex skeletal tissues first form in the embryo? And how do these tissues form so rapidly? The answers to these questions are quite complex. At the molecular level, a variety of proteins (bioactive factors) must be made and sequentially presented to a large number of specifically positioned undifferentiated progenitor cells; the large number of progenitor cells probably accounts for the rapidity of tissue formation. These bioactive factors function at the cell surface to occupy receptors and inside the cell as obligatory and necessary transcription factors. What is clear is that no one factor can trigger the entire sequence or can do it all, and that numerous diverse factors are required. For example, in transgenic knockout mice, the absence of the myogenic transcription factor MyoD or myf5 does not affect embryonic myogenesis but does affect

rib formation (in the case of myf5).[3,29] Likewise, the knockout of bone morphogenetic protein 5 (BMP5), a member of the transforming growth factor β (TGFβ) superfamily, causes the short ears phenotype in which short ears (no ear cartilage), absence of the xiphoid cartilage, and the absence of a small bony protrusion on two lower back vertebrae are the prominent malformations.[19] Clearly, MyoD, myf5, and BMP5 are powerful, highly specific effector molecules, but are only single notes in a symphony of signals that affect the appropriate responding cells to stimulate them to form an embryonic tissue.

The key component to rapid and successful embryonic tissue formation is the presence of sufficient numbers of responsive progenitor cells. Not only are the tissues relatively small, in a mass sense, in embryos, but also they have relatively very high titers of embryonic undifferentiated progenitor cells capable of responding to sequential cueing. With this logic as a background, the authors would suggest that the amount and type of tissue repair and regeneration in the adult also must have, as a rate limiting parameter, the number of progenitor cells and the identity of the signaling molecules in the neighborhood of the tissue injury and subsequent repair process.

CARTILAGE REPAIR AND REGENERATION

With regard to tissue repair and regeneration, a second principle must be recognized: tissues with high cell turnover rates (meaning replacement of expired cells with appropriate mature cells) have an improved capacity to regenerate damaged tissues. Bone, liver, and intestine are good examples of tissues with relatively high turnover rates that are capable of rapid and successful repair and regeneration; cartilage and nerve are examples of tissues with relatively low or no turnover and, thus, do not repair or regenerate. For emphasis, cartilage has been reported to have a very slow turnover capacity at the cellular and molecular level. It has been said that cartilage is a tissue that is incapable of repairing itself.[17] Specifically, when partial thickness (only penetrating cartilage) lesions or full thickness (osteochondral) defects are made in femoral or tibial surfaces, the expectation is that neither of these defects will repair.[13,18,26] This turns out to be a generalization which is not correct in that several reports indicate that small full thickness lesions are capable of fully repairing themselves; however, the actual percent of such successful repairs is relatively low (at the 10%–20% level) and only can be observed in relatively young animals.[13,26] This is to be compared with the repair of bone that heals or regenerates rapidly in young animals and also heals and regenerates in old animals after a characteristic lag period that is required to successfully form a repair blastema spanning the bone break. The puzzle is that cartilage which does not repair itself and bone which does repair itself are derived from a common progenitor cell. Such osteochondral progenitor cells have been isolated from adult marrow or periosteum.[1,2,7,9,12,14,15,20,24,25] In addition, cartilage and bone are embryologically and developmentally linked through common progenitors, not to mention the linkage in the growth plate where endochondral bone forms as the cartilage tissue is replaced by vasculature and marrow and eventually bone.[5,8]

Given this intimate association of cartilage and bone, the issue is: why does bone regenerate but not cartilage? What are the principles of bone regeneration and repair and can such general principles be applied to engineering the regeneration and repair of damaged cartilage in adult organisms?

BONE REPAIR AND REGENERATION

When a fracture occurs, the following sequence of events can be observed: The fracture disrupts the vasculature and a rapid, intense inflammatory response brings a spectrum of cells and molecules to the fracture site. The site becomes segregated from the surrounding tissue and the

accumulated inflammatory cells simultaneously decalcify the broken bone ends and provide an environment for mesenchymal progenitor cells (which are present at the site or which are chemoattracted) to divide rapidly and form a repair blastema that spans the break. In the case of an unstable fracture, the accumulated progenitor cells predominantly differentiate into cartilage. The cartilage tissue spans the fracture site whereas periosteal osteoprogenitor cells differentiate into osteoblasts and fabricate a solid bony bridge that represents the outside of the callus. The internal cartilage progresses to form calcifying hypertrophic cartilage that is invaded by vasculature and marrow, bringing a fresh supply of osteoprogenitor cells that differentiate into osteoblasts and fabricate bone onto the remnants of the calcified cartilage scaffolding. Thus, the repair blastema is converted into a bony span between the bone ends; this large bony repair tissue then remodels based on mechanical signals from normal load bearing. The long term result is the regeneration of the bone with no evidence of discontinuities between the host and repair tissue.

The following key steps are involved in bone repair and regeneration: First, the site, in response to the fracture, is flooded with inflammatory cells; this entire zone is compartmentalized and separated from surrounding tissues and vasculature. Second, the site responds to inflammatory factors by partially digesting and simplifying the host tissue and providing growth factors for reparative cells. Third, the accumulation of reparative cells, either from the site itself or from a distant source, occurs and their rapid numerical expansion is observed. Fourth, the reparative cells consolidate and group into packages of differentiating cells. The site cueing directs lineage entrance and progression of the temporary reparative tissue in an embryonic like sequence. And last, the differentiation of these cells results in the synthesis of bone and its physical, chemical, and functional integration into host tissue that has been digested partially to prepare it for molecular integration with the repair tissue. The maturation and mechanical sculpting of the repair tissue brings it in line with the tissue morphology of the host.

Cartilage Repair

With these key elements of bone repair in mind, the authors have studied the detailed cellular events involved in the repair of full thickness defects in adult New Zealand White rabbits. Below are presented selective observations from several hundred rabbit knees (Table 1) that have been analyzed using morphologic and mechanical criteria.[11,16,21,27,33]

Full thickness defects of critical size do not regenerate surface cartilage.[4,27,30] Such empty defects were analyzed in a temporal sequence at 1, 3, and 6 months. At the 6 month time point, the empty defect has more or less filled with bony tissue at the base, but the top was fibrous and housed noncartilaginous tissue as seen in Figure 1A, B. The sequential fill of this empty defect was slow but occurred in a stepwise fashion, as histologically shown in Figure 1C, D and schematically shown in Figure 2. Early in the repair process, at the base of the defect, marrow was observed to bubble up, seal off, and form a small focus of cartilaginous tissue (Fig 1D) that became hypertrophic, and was replaced by vasculature and marrow and, eventually, bone in an endochondral sequence. In a stepwise fashion, such eruptions of marrow from the base, probably stimulated by mechanical factors associated with the full weightbearing of the animal, involved the stepwise and sequential accumulation of marrow with subsequent cartilage formation and then, eventually, bony fill. Unfortunately, the top of the defect was composed of fibrous connective tissue, and hyaline cartilage was not observed in this location. It is possible that osteoprogenitor cells do reside at the surface, but that synovial fluid ambience is not appropriately chondrogenic?

In contrast, small noncritical size full thickness defects can regenerate in a low percentage of cases.[4,27] This suggests that the probable mechanism for this is the initial eruption of marrow into the empty defect

TABLE 1. Articular Cartilage Repair

Group	Defect Size	Condyle	Unilateral or Bilateral	Number	Harvest	Reference
Full thickness	1.5 × 3 mm depth	Medial or lateral	Unilateral	45	2 days, 17 weeks	27
Full thickness	2.7 diameter × 3 mm	Medial or lateral	Unilateral	45	2 days, 17 weeks	27
Full thickness	3 × 6 × 3 mm	Medial	Bilateral	49	2, 4, 12, 24 weeks	33
Full thickness + collagen gel	3 × 6 × 3 mm	Medial	Bilateral	19	2, 4, 12, 24 weeks	33
Full thickness + mesenchymal stem cells (marrow)	3 × 6 × 3 mm	Medial	Bilateral	31	2, 4, 12, 24 weeks	33
Full thickness + mesenchymal stem cells (periosteum)	3 × 6 × 3 mm	Medial	Bilateral	31	2, 4, 12, 24 weeks	33
Full thickness	3 × 6 × 3 mm	Medial	Bilateral	18	4, 12, 24, 48 weeks	34
Full thickness	3 × 6 × 3 mm	Patellar groove	Bilateral	21	4, 12, 24, 48 weeks	34
Full thickness + allogeneic chondrocytes	3 × 6 × 3 mm	Medial	Bilateral	48	4, 12, 24, 48 weeks	34
Full thickness + allogeneic chondrocytes	3 × 6 × 3 mm	Patellar groove	Bilateral	48	4, 12, 24, 48 weeks	34
Full thickness + mesenchymal stem cells (marrow)	3 × 5 × 3 mm	Medial	Unilateral	12	2, 4, 12, 24 weeks	9
Full thickness + trypsin + mesenchymal stem cells (marrow)	3 × 5 × 3 mm	Medial	Unilateral	16	2, 4, 12, 24 weeks	9
Full thickness + trypsin	3 × 5 × 3 mm	Medial	Bilateral	15	2, 4 weeks	21
Full thickness + chymopapain	3 × 5 × 3 mm	Medial	Bilateral	15	2, 4 weeks	21
Full thickness + hyaluronidase	3 × 5 × 3 mm	Medial	Bilateral	15	2, 4 weeks	21
Full thickness + chondroitinase ABC	3 × 5 × 3 mm	Medial	Bilateral	15	2, 4 weeks	21
Full thickness	3 × 5 × 3 mm	Medial	Bilateral	4	2, 4 weeks	21
Full thickness	4 diameter × 3 mm	Medial	Unilateral	23	3, 6 weeks	11
Full thickness	2.7 mm diameter × 3 mm	Lateral	Bilateral	24	1, 2, 4, 12 weeks	11
Partial meniscectomy + 6 week, full thickness	2.7 mm diameter × 3 mm	Lateral	Bilateral	24	1, 2, 4, 12 weeks	11
Partial meniscectomy + full thickness	2.7 mm diameter × 3 mm	Lateral	Bilateral	24	1, 2, 4, 12 weeks	11
Partial meniscectomy, full thickness	4 × 3 mm	Medial	Unilateral	13	6 weeks	11

TABLE 1. (Continued)

Group	Defect Size	Condyle	Unilateral or Bilateral	Number	Harvest	Reference
Partial meniscectomy + 6 weeks, full thickness	4 × 3 mm	Medial	Unilateral	12	6 weeks	11
Partial meniscectomy, full thickness + gel	4 × 3 mm	Medial	Unilateral	12	3, 6 weeks	11
Partial meniscectomy + 6 weeks, full thickness + gel	4 × 3 mm	Medial	Unilateral	12	3, 6 weeks	11
Partial meniscectomy, full thickness + chondrocytes	4 × 3 mm	Medial	Unilateral	12	3, 6 weeks	11
Partial meniscectomy + 6 weeks, full thickness + chondrocytes	4 × 3 mm	Medial	Unilateral	12	3, 6 weeks	11
Partial meniscectomy, full thickness + mesenchymal stem cells (marrow)	4 × 3 mm	Medial	Unilateral	12	3, 6 weeks	11
Partial meniscectomy + 6 weeks, full thickness + mesenchmal stem cells (marrow)	4 × 3 mm	Medial	Unilateral	12	3, 6 weeks	11
Full thickness: composite gel with chondrocytes + ceramic with mesenchymal stem cells	3 × 6 × 3	Medial	Bilateral	24	4–30 weeks	
Full thickness with polylactic + mesenchymal stem cells	3 × 6 × 3	Medial	Unilateral	16	4–30 weeks	
Shallow: collagen gel + chondrocytes + dexon cover sheet	5 × 5 × 2 mm	Patellar groove	Bilateral	62	1–32 weeks	
Shallow: hardened collagen gel + chondrocytes	5 × 5 × 2 mm	Patellar groove	Bilateral	46	1–32 weeks	
Full thickness + mesenchymal stem cells + TGFβ	3 × 6 × 3 mm	Medial	Bilateral	12	4, 8, 14 weeks	
Full thickness + mesenchymal stem cells + bFGF	3 × 6 × 3 mm	Medial	Bilateral	12	4, 8, 14 weeks	
Total				811		

TGFβ = transforming growth factor β; bFGF = basic fibroblast growth factor.

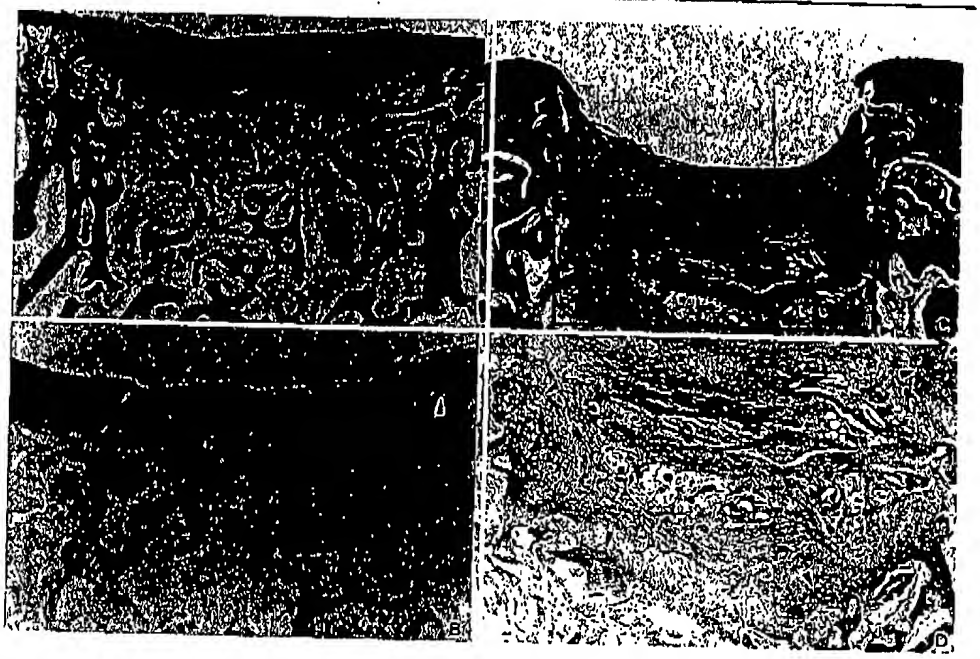

Fig 1A–D. (A) Full thickness empty defects measuring 4 mm in diameter by 3 mm deep were prepared in the medial femoral condyle of young adult New Zealand White rabbits without (B,C,D) or with a partial medial meniscectomy following the procedure of Moskowitz et al[22,23] all stained with toluidine blue. The animals were sacrificed 4 to 6 weeks after creating the defects and the condyles prepared for routine histologic study. The most rapid and complete self repair observed in 94 empty, full thickness defects is viewed in this section. The subchondral bone was regenerated almost completely to the original height. The tissue at the top is a fibrocartilage (magnification ×20). (B) Typical of the partial meniscectomy joint in this rabbit model of osteoarthritis was the relatively rapid regeneration of the subchondral bone with a surface layer of predominantly fibrous tissue at the 6 week sacrifice time. The arrowhead identifies the right edge of the defect (magnification ×20). (C) Typical of one of the slowest to fill empty defects in a partial meniscectomized knee, this section looks like the average empty defect in a normal joint (magnification ×20). (D) Higher magnification from the bottom center of C (boxed area; magnification ×35). Cartilage (arrowheads) is presumed to originate from eruptions of marrow into the fibrous repair tissue. This cartilage will be replaced by bone in an endochondral sequence to fill the entire defect by 6 months.

which causes marrow to fill completely the defect with osteoprogenitor cells that aggregate and multiply and then differentiate into cartilage, and eventually this cartilage is replaced by bone at the base.

To test this suggestion, full thickness defects were created in the distal femoral medial condyle that were then filled with freshly harvested marrow. Animals were analyzed morphologically at 6 and 12 weeks. Initial observations include the rapid and impressive fill of the defect with accumulation of cartilaginous cells that rapidly were replaced by bone. Characteristically, there was a thick and uniform fibrous layer at the top of the defect that may contain numerous osteoprogenitor cells. By 12 weeks, bone has completely filled this defect well past the cartilage subchondral bone line and some of the cells at the top were clearly hyaline cartilage like. However, fibrillation and destruction of this tissue was evident and it is as-

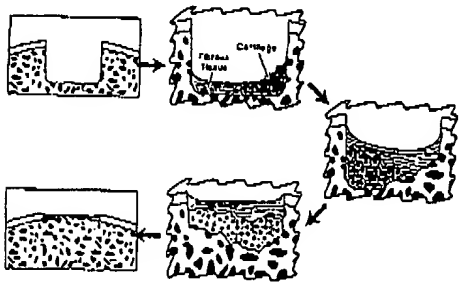

Fig 2. Schematic representation of marrow eruptions into a full thickness empty defect that will be replaced endochondrally by bone. The replacement of the cartilage by vasculature, marrow, and then bone was followed by another marrow eruption until the defect was filled completely by bone with a covering of fibrous tissue.

sumed that these joints would be considered failures by 6 months. Thus, implanting whole marrow to fill completely the defect from the beginning of the repair cycle provides sufficient osteochondral progenitor cells to fill most of the defect volume with cartilage producing cells. However, there are probably not enough of these progenitor cells to fill completely the defect to the top with functional hyaline cartilage. In addition, it is suspected that the relative slowness of the fill of the defect eventually results in mechanically inappropriate surface tissue leading to fibrillation and eventually tissue failure.

Partial meniscectomy provides a model for initiating experimental degenerative osteoarthritis. Partial meniscectomy creates an unstable joint, that by 6 weeks shows signs of pitting and osteophyte formation which are statistically distinctive from that observed in the unoperated controls.[22,23] By 12 weeks, morphologically apparent osteoarthritis, noted by pitting and osteophyte formation, can be documented clearly in the unstable joint. One can interpret this sequence to be a combination of destructive and constructive signals. The response of the joint to the instability is to attempt to remodel the joint by breaking down and rebuilding specific tissues: breaking down cartilage and building osteophytes perhaps to act as mechanical stabilizers. This is comparable with guided tooth movement caused by an appliance strain; in this case, bone is resorbed to relieve the compressive strain at the front of the tooth while at the back, bone is filled in synthetically in response to tensile load to allow the tooth to be anchored firmly. In this latter case, destructive (resorptive) events and constructive (new bone synthesis) events are coupled. The partial meniscectomy causes mediators and enzymes to enter into the joint; these bioactive factors function to break down cartilage and provide stimulation for new bone formation in the form of osteophytes. The formation of the osteophytes may serve to stabilize the joint by constructing bony protrusions to interface mechanically with the opposing structure. Although the formation of osteophytes may be a response to mechanical abnormalities, biologic events also may drive the process. For example, when large amounts of TGFβ are injected directly into a normal joint, osteophytes also can be observed.[32] Thus, the unstable joint may induce the synthesis or release of an agent such as TGFβ into the joint to stimulate the construction of new tissues. Importantly, bone, itself, is a repository of bioactive factors and responsive cells.

An important issue is whether an empty full thickness, critical size defect will undergo repair in the meniscectomized knee. The authors have studied the repair sequence of such defects using a semiquantitative histologic scoring system with perfect repair equal to 16 as summarized in Tables 2 and 3.[11] These data show a relative improvement in the histologic score in the meniscectomized knee; the apparent improvement occurs when the meniscectomy is completed 6 weeks before placing the critical sized defect in the femoral condyle. In the ensuing 6 weeks, the subchondral bone is regenerated rapidly compared with the empty defect in

the normal knee or the empty defect in a knee that had the meniscectomy concurrent with placement of the defect. Thus; the authors would suggest that the constructive agents in the unstable joint may accelerate the bony fill of the defect.

Several observations from all of the above are clear: Marrow contains progenitor cells for repair and regeneration of these defects.[2,4,30,33] The amount of marrow is crucial to the degree of repair and regeneration.[33] The age of the animal and, thus, age related quantity of marrow derived progenitor cells are crucial to the extent and rate of defect repair.[15] The cytokines in the joint of stable or unstable knee influence the rate and extent of repair and regeneration.[17,22,23,32] And last, the size of the defect is a determinant of repair and regeneration.[4,27,30]

Chondrocyte Implantation

Chondrocytes in culture[31] have been shown to faithfully produce hyaline cartilage (that is, they are articular chondrocytes) but also can be encouraged to lineage progress to hypertrophic chondrocytes (which, in turn, stimulate the invasion of vascular and marrow elements with subsequent endochondral differentiation of bone[10]). Therefore, it is not unreasonable to use articular chondrocytes to fill full thickness condyle defects in an attempt to regenerate skeletal tissues in these wounds. Obviously, the top of such chondrocyte filled defects should be hyaline cartilage because others have shown that, when articular chondrocytes are taken from in vivo and placed into culture, they continue to fabricate exactly the same extracellular matrix as they did when they had been in in vivo locations.[28,31]

Wakitani et al[34] have placed allogeneic chondrocytes into a full thickness defect in a type I collagen carrier in the knee in the patellar groove and, further, recently have done the same type of implantation in critical size defects in the medial femoral condyle. Indeed, these allogenic chondrocytes rapidly fabricate a rich cartilaginous matrix and completely fill the entire defect. However, even after 6 months, these cells appear to be stable and have not continued down the lineage to form hypertrophic cartilage that is replaced endochondrally by subchondral bone (Fig 3A). Moreover, the integration of host and donor tissue is never observed (arrowheads, Fig 3A). Thus, an unintegrated plug of hyaline cartilage fills the entire defect from the bony base to the top of the articular zone. Even though the cartilage at the top is, initially, hyaline cartilage, there is a mechanical mismatch between host cartilage which sits on its subchondral bone base, and donor cartilage, which is a thick plug occupying the depth of the entire defect. This mismatch eventually leads to fibrillation of the surface cartilage and failure of the implant. Importantly, although this hyaline cartilage forms rapidly, it does not integrate with the host cartilage. Thus, cartilage and chondrocytes fixed at one lineage stage are not suitable for these full thickness defect sites; long term failure can be expected although a short term fill is observed. This mismatch is to be compared with that of the repair tissue observed when mesenchymal stem cell preparations are implanted into the full thickness defect as is discussed in the next section (Figs 3B–D). Figures 3C and 3D focuses on this interface by comparing the polarized light (Fig 3C) and stained (Fig 3D) images of cartilage repair viewed at 3 months after implantation (the arrowhead marks the edge of repair and host tissue).

MESENCHYMAL PROGENITOR CELLS

From the above, the authors reasoned that the key to cartilage repair was the presence of enough reparative cells in the defect. Because the defect encompasses the subchondral bone and the cartilage, the reparative cells must be so situated as to provide repair for both of these very different types of skeletal tissue. Going back to the developing embryo for clues, the authors realize that in the initial joint formation, the articular cartilage to bone interface formed from a solid

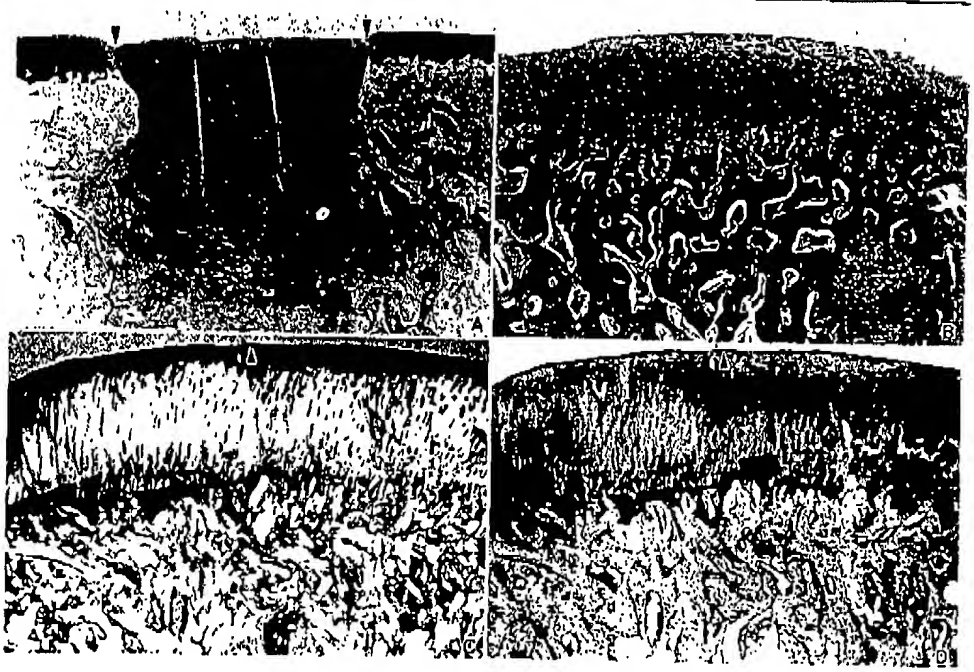

Fig 3A–D. Cell based cartilage repair. (A) Allogenic articular chondrocytes are harvested from 1 to 2 month old New Zealand White rabbits, incorporated into a Type I collagen gel and implanted into a full thickness defect comparable with those shown in Figures 1, 2, 4, and 5. The section pictured here was harvested from an animal 6 months after implantation; the arrowheads indicate the edge of the defect that is filled with healthy articular hyaline cartilage throughout most of the depth of the defect (stain, toluidine blue; magnification ×20). (B) Autologous marrow derived mesenchymal stem cells were incorporated into a Type I collagen gel and delivered to a full thickness defect in an adult rabbit; the animal was sacrificed 4 weeks later and the medial femoral condyle prepared routinely for histologic study. Most of the depth of the defect shows regenerated subchondral bone, that has formed endochondrally; a mass of untransformed cartilage can be seen on the right next to B; this cartilage presumably will be replaced endochondrally by bone (stain, toluidine blue; magnification ×30). Polarized (C) and light (D) microphotographs show autologous mesenchymal stem cells that were implanted 6 months before sacrifice in a full thickness defect on the medial femoral condyle of an adult rabbit; the defect was pretreated with a dilute trypsin solution before implanting the mesenchymal stem cell and gel composite. The arrowhead in Figure 3C is next to the edge of the defect with repair cartilage to the right and host cartilage to the left. The repair cartilage and bone matched the images of the host cartilage and bone (stain, toluidine blue; magnification ×100).

embryonic cartilage structure called the cartilage model. This embryonic cartilage model of the distal femoral head, for example, keeps the surface chondrocytes at the articular stage of development while the central chondrocytes can be observed to lineage progress to the hypertrophic stage where they are replaced by marrow and vasculature and a second wave of progenitors differentiate into bone fabricating osteoblasts. These newly formed osteoblasts construct bone in the space previously occupied by the embryonic cartilage with the overlying articular cartilage layer remaining intact. Thus, to repair or regenerate a full thickness defect, a bulk of progenitor cells must be delivered into the entire defect and these must differentiate rapidly into the appropriate solid plug

TABLE 2. Partial Meniscectomy + Full Thickness Defects: Histologic Grades

Group (n = knees)	*1 week*	*2 weeks*	*1 month*	*3 months*
Full thickness (6)	0.2	1.9	6.3	6.9
Partial meniscectomy, full thickness (6)	0.2	2.9	8.2	7.0
Partial meniscectomy + 6 weeks, full thickness (6)	0.2	6.4	7.4	5.9

of embryonic chondrocytes that then can progress at the top to articular and at the bottom to hypertrophic chondrocytes.

As reviewed above, whole marrow contains such progenitor cells that differentiate rapidly into chondrocytes when implanted into a full thickness defect. However, because these defects do not regenerate completely, it is probable that an insufficient number of such progenitors are delivered into the defect when using the whole marrow specimen. Thus, the authors have isolated progenitor cells from bone marrow or periosteum as rich repositories of osteochondral progenitor cells that the authors call mesenchymal stem cells[6,9,14]; these cells have

TABLE 3. Semiquantitative Histologic Grading Scale (Maximum = 16 Points)

Grading Scale	Points
Cell Morphology	
Normal	4
Mostly hyaline cartilage	3
Mixed hyaline and fibrocartilage	2
Mostly fibrocartilage	1
Some fibrocartilage, mostly nonchondrocytic cells	0
Reconstruction of subchondral bone	
Normal	3
Reduced subchondral bone reconstruction	2
Minimal subchondral bone reconstruction	1
No subchondral bone reconstruction	0
Matrix staining	
Normal	4
Slightly reduced	3
Reduced	2
Significantly reduced	1
No staining	0
Filling of defect	
100%	2
50 or 150% (overfill)	1
0%	0
Surface regularity	
Regular, smooth	1
Irregular	0
Bonding	
Both graft edges bonded	2
One graft edge bonded	1
Neither edge bonded	0

been culture expanded to increase their number and have been implanted into full thickness defects in the medial condyle of the distal femur of the adult rabbit.[7,21,23] The logic is that these osteochondral progenitor cells will lineage progress to hypertrophic cartilage at the base and give rise, eventually, to bone whereas those at the top, in contact with synovial factors, will remain as articular chondrocytes.

It has been shown previously that such isolated and culture expanded mesenchymal stem cells have osteochondral potential when tested in three separate in vivo assays.[7,14,15,24,25] The difficulty was to decide how to deliver these cells to the full thickness cartilage defect. In this regard, it was observed that all primitive embryonic mesenchyme is housed in a very low percentage, very loose (highly hydrated) Type I collagen matrix. Thus, passaged, autologous marrow or periosteal mesenchymal stem cells in a 0.15% Type I collagen gel as a delivery vehicle have been used; the experimental flow is shown in Figure 4.[33] Animals were sacrificed at 1, 2, and 3 weeks and at 1, 3, and 6 months and histologic (Figs 5 and 6) and biomechanical testing and analysis of the repair tissue was performed. Figure 3B and Figure 6B show the results 1 month after implantation. The resurfacing of the defect with articular cartilage with the regeneration of the subchondral bone up to the natural cartilage bone junction was observed. Initially, it was presumed that the mesenchymal stem cells at the base differentiated directly into osteoblasts that fabricated the subchondral bone and directly into surface articular chondrocytes. However, with a more detailed analysis at all of the initial time points, it became clear that implanted cells recapitulated the embryonic sequence. Initially, all of the implanted mesenchymal stem cells rapidly differentiated into embryonic cartilage. The chondrocytes at the base of the defects lineage progress to hypertrophic chondrocytes that were replaced by vasculature, marrow and fresh osteochondral progenitors that differentiated into osteoblasts to form bone on the remnants of the calcified hypertrophic cartilage (Figures 6A, 6B, 6E and 6F). Thus, new bone was derived from host cells whereas the articular cartilage developed from the initially implanted mesenchymal stem cells. The top cartilage remains as articular cartilage, probably from exposure to synovial factors, whereas the subchondral bone continued to change until at 6 months

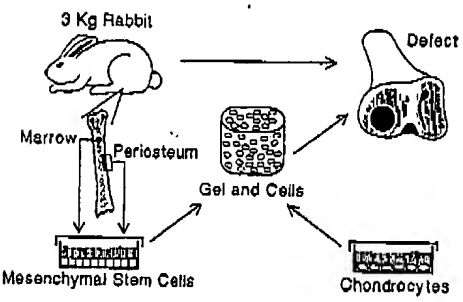

Fig 4. Marrow or periosteum was obtained from an adult New Zealand White rabbit, the mesenchymal stem cells isolated and culture expanded, a large, full thickness defect prepared in the medial femoral condyle, and either autologous mesenchymal stem cells or allogenic chondrocytes were incorporated into a Type I collagen gel and implanted into the defect.

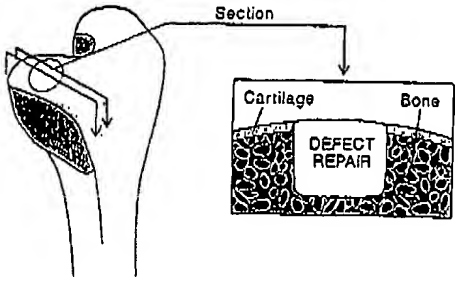

Fig 5. A large full thickness defect was prepared in the medial femoral condyle of adult New Zealand White rabbits. After sacrifice, histologic sections were prepared through the defect to view the entire defect and surrounding host tissue.

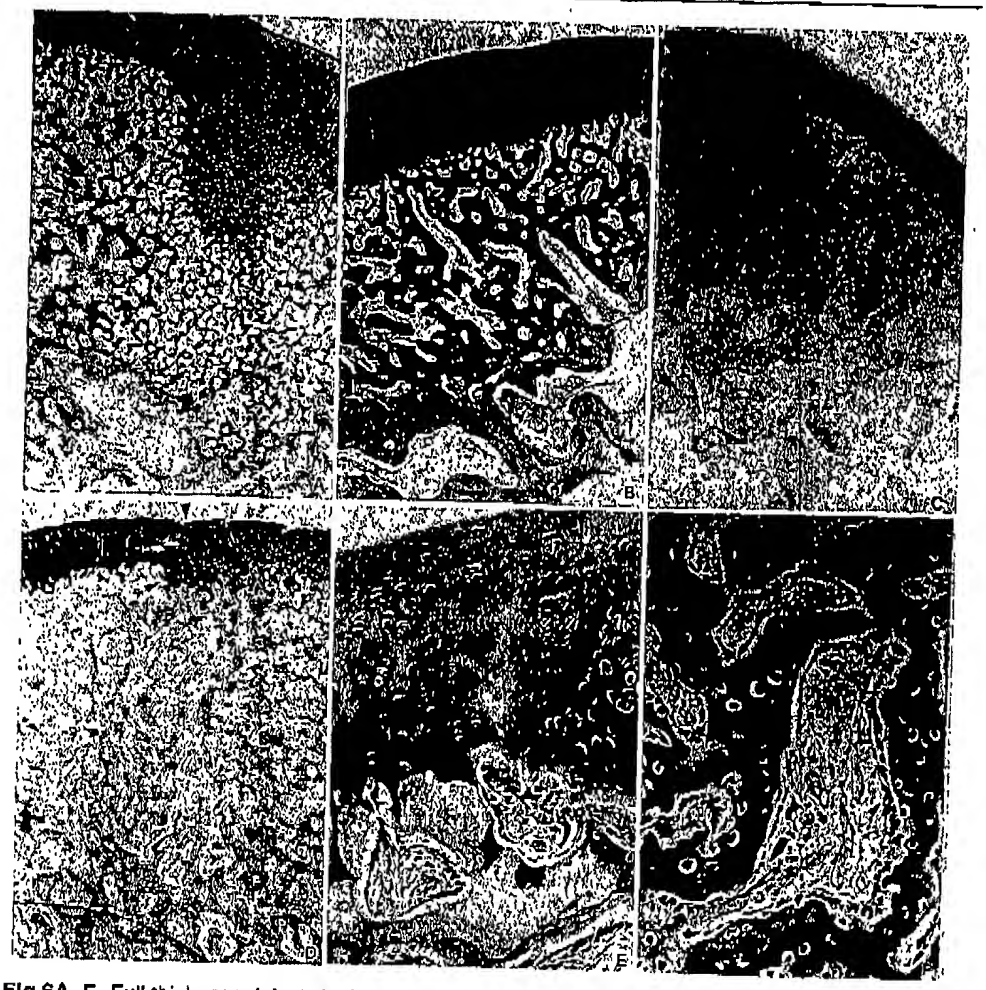

Fig 6A–F. Full thickness defects in the medial femoral condyle were filled with culture expanded autologous mesenchymal stem cells in a Type I collagen gel. The animals were sacrificed at (A) 3 weeks, (B, D, E, F) 1 month, or (C) 6 months and the knees were processed routinely for histologic study. A. All of the cells in the defect have differentiated into embryoniclike chondrocytes that produce a rich, cartilaginous matrix. From the bony walls of the defect inward and upward, the cartilage was being replaced and endochondral bone was forming (stain, toluidine blue; magnification ×30). A higher magnification view of the lining osteoblasts fabricating this new bone is seen in E and F. One month after implantation, the endochondral bone formation produces trabecular bone of a different density than the host bone; see B. (stain, Mallory/Heidenhain; magnification ×40) comparing the bone with that in C. C. At 6 months after implantation, the bone density and articular cartilage were indistinguishable from host (stain, toluidine blue; magnification ×40). D and E. Various degrees of host:regenerate integration of cartilage can be observed. D shows a mixture of highly cellular cartilage and repair tissue at the junction of host and repair tissue (arrowhead; stain, toluidine blue; magnification ×20) whereas (E) shows the relatively intact host cartilage on the left and repair cartilage on the far right. Between these two zones was an interface zone which seems to be continuous with the repair and host zones and which clearly contains host cells which are cloning as often is seen in repairing osteoarthritis (stain, toluidine blue; magnification ×250). (F) Stain, Mallory/Heidenhain; magnification ×250. Arrowheads point to sheets of bone forming osteoblasts.

(Fig 6C), it resembled the density of host bone. This stepwise sequence is summarized in the drawings found in Figure 7.

Although the regeneration was swift and impressive, 20% to 100% of the circumference of the regenerated cartilage was not integrated physically with host tissue, although, even when integrated, the repair and host tissue interface could be discerned (Fig 6D, E). This discontinuity between regenerate and host tissue looked, in some cases, like a simple knife laceration of the cartilage, that is known to never heal and can cause long term failure of the joint surface.[17] From high resolution light and electron microscopy of the junction of host and repair tissue, the impressive molecular architecture of the host defect cartilage edge with little or no disruption in matrix morphology was observed. This can be interpreted to indicate that in creating the defect, the cut cartilage tissue rapidly seals itself and preserves its integrity. In contrast, as reviewed above, the fractured ends of bone facing the open wound are attacked rapidly by enzymes and reagents involved in the initial and massive inflammatory response. The result is the decalcification and partial digestion of the bone matrix with remnant collagenous scaffolding remaining.

Again with this fracture model in mind, enzymatic digestion of the cartilage wound surface of the full thickness defect was chosen as a way of opening the cartilage matrix by denuding it of proteoglycans; the speculation was that this then would allow the inter-

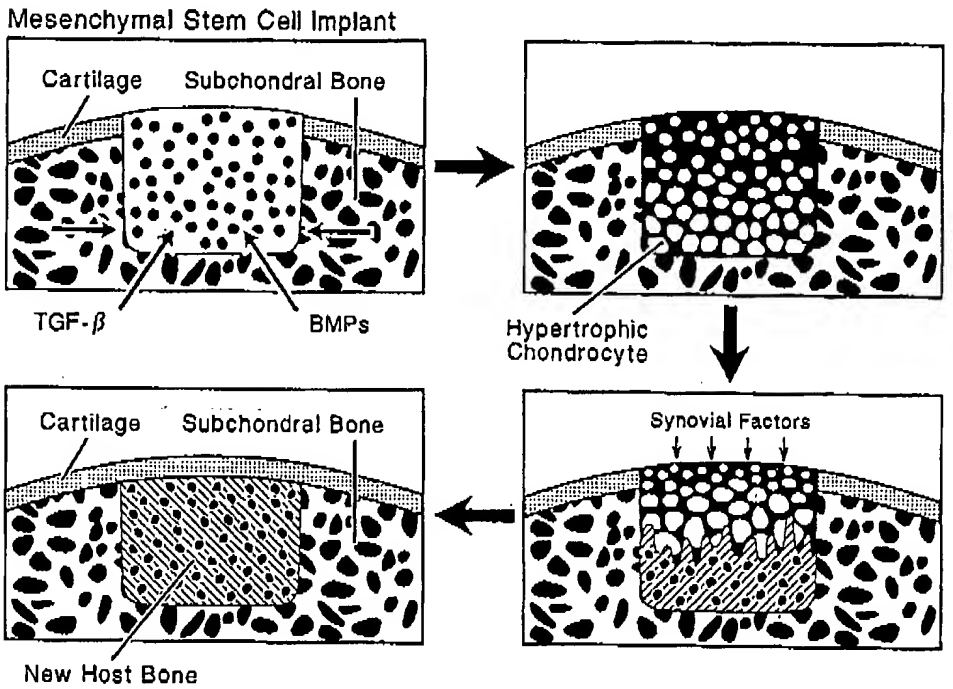

Fig 7. Schematic drawings showing the sequence of events during the first 4 weeks, from the time of implantation of mesenchymal stem cells in a collagen gel (upper left panel) to the time of replacement of the resulting cartilage by host bone covered by donor articular cartilage. TGFβ = transforming growth factor beta, and BMPs = bone morphogenetic proteins.

digitation with newly synthesized matrix components from the implanted and repairing tissue. Numerous enzymes were screened that were chosen to leave the collagenous scaffolding of cartilage relatively intact while clipping various matrix macromolecules.[21] The optimal reagent was found to be a dilute trypsin solution, which clips the core protein of aggrecan and other matrix proteoglycans that are, thus, lost from their anchorage sites in the matrix although leaving the Type II collagen scaffolding. A detailed concentration array was tested and 0.005 mg/mL trypsin soaked into a piece of filter paper that was laid into the defect for 5 minutes before mesenchymal stem cells delivered in a Type I collagen gel were implanted, was found to be optimal for maximal integration of host and repair tissue viewed at 3 to 6 months after implantation.

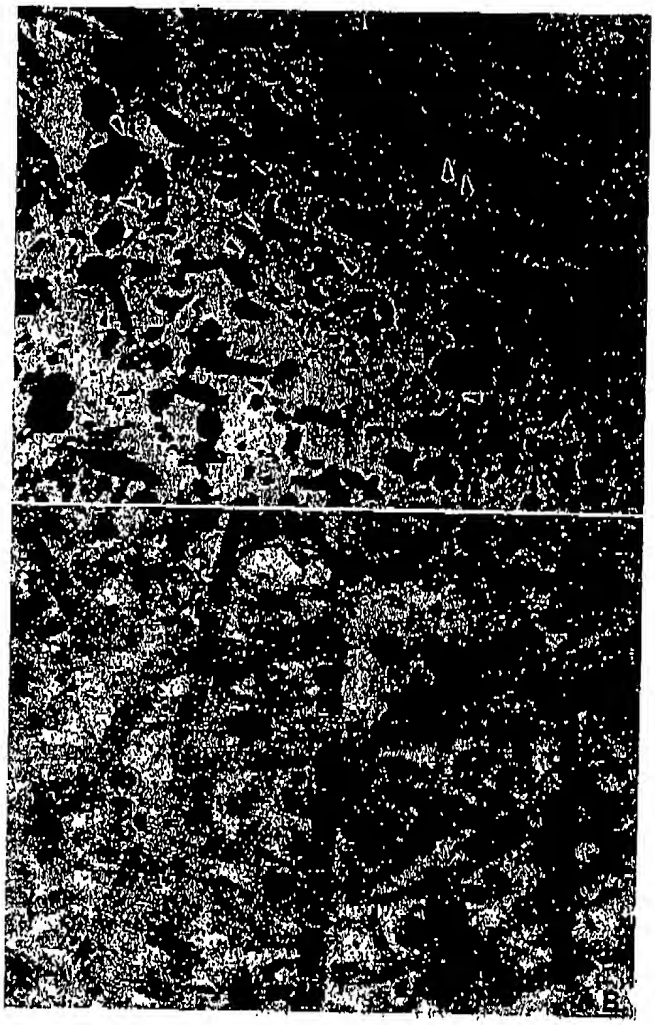

Fig 8A–B. Full thickness defects were prepared as in Figures 4 and 5 and pretreated with 0.005 mg/ml trypsin for 5 minutes, washed with saline solution and fixed with 2% glutaraldehyde, 0.7% ruthenium hexamine trichloride, 50 mm sodium cacodylate, ph 7.4.[16] Tissues were postfixed in 1% osmium tetroxide, 50 mm sodium cacodylate, and en bloc stained with 2% uranyl acetate (A). Untreated cartilage matrix near the edge of the defect at a locale identical to (B). Trypsin treated. Arrowheads point out the electron dense ruthenium hexamine trichloride that binds and collapses proteoglycans. The empty spaces in (A) are interpreted to represent the volumes occupied by water and proteoglycan composites although in (B), these have collapsed presumably because of the proteolysis of the core proteins and subsequent loss of water structuring glycosaminoglycans from the matrix. Original magnification: (A) ×21,000, (B) ×52,000.

As part of the analyses of the effect of pretreatment of the defect with trypsin, untreated and freshly treated defects were fixed and embedded and the cartilage matrix analyzed with the aid of the electron microscope. In Figure 8, the cartilage matrix is visualized and the single clear impression in comparing Figure 8A with 8B is that trypsin treatment causes the loss of proteoglycans as indicated by a reduction in the quantity of ruthenium red granules (arrowheads) with the subsequent collapse of the matrix. The protease treated matrix is observed to have much less structured water and, thus, this matrix can, presumably, be infiltrated more easily with newly synthesized matrix from the expanding repair tissue. These suggestions are amenable to experimental verification.

SUCCESSFUL CARTILAGE REGENERATION

From the above, several important concepts emerge: First, successful repair of skeletal tissue defects requires that a recapitulation of embryonic events be engineered. The key component is the number of progenitor cells brought to the repair site. Clearly, when bone breaks such repair cells arrive to form the repair blastema from the periosteum, marrow, and peripheral sources. Because cartilage has no vascular supply or easy access to such progenitors, such reparative cells must be provided, in bulk, at the cartilage defect site. Interestingly, full thickness, relatively small lesions in very young animals can repair themselves completely probably because the marrow that rapidly fills these small defects has a relatively very high titer of such reparative cells and signaling molecules. Second, the right number of reparative cells must be delivered initially and must be in a suitable and appropriately flexible delivery vehicle. A very dilute Type I collagen gel that mimics embryonic mesenchyme appears to be quite suitable in this regard. Lastly, the surrounding host tissue must be prepared for molecular interdigitation with the rapidly developing repair tissue in the defect. This preparation must negate the natural tendency of the tissue to seal itself and must denude the host matrix of anchored, water structuring and sealing molecules that inhibit the interdigitation of newly synthesized molecules from the repair tissue. Such treatment must create a more highly hydrated tissue with a relatively dilute matrix to match the embryonic like matrix that first forms in the recapitulation sequence observed in the repair tissue.

Taken together, for skeletal tissues, hard and soft, the term repair is not a satisfactory term or concept. Given the recapitulation of embryonic events and the mechanisms that govern such a progressive sequence, these events, by necessity, must be termed true regeneration. The concepts which, thus, must govern the logic and, therefore, the tissue engineering motifs, are those of embryonic development, not patch it, fix it up repair. A scar in skeletal tissues is a mechanical discontinuity in a material sense and a location for future mechanical failure. Regeneration events involve the orderly sequences of change to bring about adult tissue formation and must also involve integration with the surrounding host tissue. Thus, the use of these concepts and principles is necessary to bring about the full regeneration of articular cartilage with its subchondral bone. Having made the above argument, the authors recognize that the above puts forth a very demanding standard. Regeneration must be considered perfect repair and, indeed, the authors have selected photomicrographs to emphasize the concept of perfect repair. Perfect repair, however, is a rarity in these experiments. The standard of full regeneration is extremely demanding but should be the goal of tissue engineering logicians if they are to be clinically relevant. Refinements of the current technologies obviously are necessary.

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